cells expressing *Shaker* or increasing it in case of Kv3.1. (Support: NIH-GM030376, FWO-G025608)

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Use of Tethered Spectroscopic Probes as Chemical Calipers to Measure Molecular Distances

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Fluorescence based techniques have the advantage of being able to probe dynamic structural changes in a physiological milieu. Here we describe the development of a novel approach to estimate molecular level distances between two sites in a protein structure. Distance information was inferred by measuring accessibility of a fluorescent tag on one site to a variable length collisional quencher covalently affixed to a second site. The functionalized variable length quenchers therefore act as ''tape measures'' which can be utilized to obtain point-to-point distance information in the protein structure. We validated our approach using model proline polypeptide substrates of varied lengths (n = 6 or 10). Polyproline substrates were flanked with an N-terminal fluorophore and a C-terminal cysteine. Cysteine reactive tailed quenchers of varying lengths were attached at the C-terminal end. The quenching compounds contained two reactive tails, one that includes a maleimide group to cross-link with the free sulfhydryl of cysteine and a second that has a nitroxide radical quenching moiety, joined by a polyethylene glycol (PEG) spacer of known lengths (n = 3-8). Our results demonstrate that variable length tethered quenchers are able to estimate molecular distances between two attachment sites by exploiting bimolecular quenching. Quenching efficiency closely matched the predicted average conformation length and sampling radius of the PEG spacer as determined by Monte Carlo simulations. Our findings establish the feasibility of using these tools to determine nanometer scale distances between two sites in a protein structure in real time.

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Ca^{2+} Dependent Activation of Large Conductance Ca^{2+} Activated Potassium (BK) Channels by Binding to the RCK1 Domain

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BK channels sense intracellular Ca^{2+} and are important modulators of muscle contraction, neuronal spike frequency adaptation, neurotransmitter release and circadian pacemaker output. The cytosolic domain (CTD) of BK channels contains two structural sub-domains, RCK1 and RCK2. Mutagenesis studies have identified a series of Asp residues in the RCK2 domain (termed as the Ca^{2+} bowl) and Asp367 in the RCK1 domain as two putative Ca^{2+} binding sites. A recently published crystal structure of the BK CTD showed Ca^{2+} binding to the Ca^{2+} bowl but, surprisingly, provided less further information about the Ca^{2+} binding site in RCK1. We have performed mutational scan in the RCK1 domain to search for the residues that are necessary for Ca^{2+} binding in addition to Asp367. Here we show that the mutations of Glu535 in the RCK1 domain produce nearly identical functional consequences on the Ca^{2+} dependent activation as the mutations of Asp367. Therefore, Glu535, same as Asp367, may be one of binding coordinators for Ca^{2+} binding in RCK1. We also show that mutations of Met513, some of which have been previously shown to reduce Ca^{2+} sensitivity, result in a different pattern of functional consequences than those of Glu535 and Asp367, which suggest that Met513 is not part of the Ca^{2+} binding site. Molecular modeling and experimental data suggest that the binding of Ca^{2+} by the side chains of Glu535 and Asp367 changes the conformation around the binding site and turns the side chain of Met513 into a hydrophobic core, thereby opening the activation gate of BK channels. We have also investigated Cd^{2+} dependent activation of BK channels and found that Ca^{2+} and Cd^{2+} interact with different sets of residues to activate BK channels.

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Calcium-Dependent Operation of the Human BK Channel Gating Ring Apparatus

Taleh Yusifov, Anoosh Javaherian, Chris Gandhi, **Riccardo Olcese**.

The large cytoplasmic C-terminal domain (CTD) of the human BK channel forms a gating ring structure composed by two tandem RCK domains serving as a signal transducer for intracellular Ca^{2+} and other ligands. However, the mechanism of the gating ring's operation remains unknown. We have used steady-state and time-resolved spectroscopy in combination with dynamic light scattering to the Ca-induced conformational changes of the purified CTD of human BK channel. The CTD domain of the human BK channel assembles as a tetrameric gating ring structure (MW~310 kDa) with a hydrodynamic radius (R_H) ~10.5 nm. In the presence of 35 μM free Ca^{2+}, R_H reversibly decreases to ~7.5 nm. The modulation of the gating ring hydrodynamic shape suggests that it undergoes Ca^{2+}-induced conformational transitions, which we further, characterized using time-correlated single-photon counting spectroscopy. Increasing free [Ca^{2+}] up to 35 μM shortened the average Trp fluorescence lifetime (τ_{avg}) of the wild-type gating ring from ~2.6ns to ~1.9ns, while the neutralization of the high-affinity Ca-binding site within RCK2 (Ca bowl, D894-898N) attenuated the effect of Ca^{2+}. Steady-state fluorescence analysis revealed that these ligand-dependent structural rearrangements of the gating ring possess strong divalent cation selectivity. The gating ring exhibited i) high-affinity Ca^{2+}-binding (K_{half_1} ~0.3μM and K_{half_2} ~4μM); ii) significantly lowered affinity for Mg^{2+} (K_{half} ~200μM) and iii) no Ba^{2+} sensitivity up to 13mM, consistent with the lack of Ba^{2+}-dependent BK channel activation. Interestingly, Ca^{2+} bowl neutralization eliminated Mg^{2+} sensing up to 12mM. In summary, under physiologically-relevant conditions, these ligand-induced conformational transitions are strongly ion-specific and associated with changes in the hydrodynamic properties of the BK gating ring and likely represent the ligand-induced molecular events underlying channel activation.

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On the Properties of the RCK1 Domain of the Human BK *(SLO1)* Channel

Taleh Yusifov, Anoosh Javaherian, Antonios Pantazis, Chris Gandhi, Riccardo Olcese.

In BK channels, four RCK1 and four RCK2 domains assemble into a cytosolic ligand-sensing superstructure known as the gating ring. While electrophysiological data suggest that both RCK1 and RCK2 contain high affinity Ca^{2+}-sensing sites, recent crystallographic data (Wu et al., 2010) has revealed Ca^{2+} binding only within the RCK2 domain. Does the RCK1 domain bind Ca^{2+}? What is the role of the high-affinity Ca^{2+} sensing residues D362/D367 (Xia, et al 2002) located in RCK1 domain? To address these questions, we expressed and purified the region of the human BK channel C-terminus corresponding to the amino acid sequence ^{322}IIE¼HDP^{667}. We have probed the structure and Ca^{2+} sensing properties of the RCK1 domain in solution, under physiologically relevant conditions. In addition to the α/β fold shared with its bacterial counterparts and human BK RCK2 domain, the RCK1 domain preferentially self-assembles into a homo-octameric structure. We recently reported that RCK1 undergoes Ca^{2+}-dependent conformational changes similarly to the RCK2 domain (Yusifov et al., 2008 and 2010). The neutralization of residues D362/D367 altered the secondary and quaternary structure of the RCK1 domain and prevented Ca^{2+}-induced structural transitions in RCK1. $^{45}Ca^{2+}$ overlay assay suggested that the neutralization of D362/367 did not abolish the Ca^{2+}-binding activity of RCK1 in the range of 2.1-115 μM free Ca^{2+}. While it cannot be excluded that D362/D367 are elements of a Ca^{2+} binding site with multiple coordinating residues, our results favor the view that D362 /D367 have a predominantly structural role. Possibly, these mutations set RCK1 domains in a conformational state that hampers the propagation of structural changes caused by ligand binding.

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Probing the Dynamic Structure of the BK_{Ca} Voltage Sensor: Relative Motion of Segments S0 & S4 During Activation

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Voltage- and Ca-activated, Large-conductance Potassium (BK_{Ca}) channel α subunits (Slo1) possess the transmembrane helix S0 at their N-terminus, which is absent in other members of the voltage-gated channel superfamily. S0 mediates the interaction between pore-forming α and modulatory β subunits, while it is thought to pack closely to voltage-sensing segment S4 (Liu *et al.*, 2010). We previously reported that conformational rearrangements relevant to voltage-dependent activation occur in the proximity of the BK_{Ca} N-terminus, by using voltage clamp fluorometry (Pantazis *et al.*, BPS meeting 2010). In this work, we have identified the principal molecular source of the fluorescence signal reported from the extracellular flank of S0 (positions 17, 18 or 19 labeled with the fluorophore TMRM) in W203, at the extracellular tip of S4: Substitution of W203 with a Valine reduced the amplitude of voltage-dependent ΔF/F reported from the extracellular portion of S0 by ≈90%. Accordingly, we demonstrate that the tryptophan side-chain is able to efficiently quench TMRM fluorescence with a Stern-Volmer constant K_{SV} = 41.3 M^{-1}. Considering this data, we suggest a model for the dynamic structure of the BK_{Ca} voltage sensor domain: at rest, S0 is in collisional proximity with S4, so that TMRM labeling S0 is quenched by W203. Upon depolarization, a relative motion between the two segments increases the distance between them, relieving TMRM quenching. Regarding the relevance of the relative motion between S0 and S4 to channel activation, we propose that S0 acts as a pivot, against which the voltage-sensitive S4 moves to actuate channel gating. We speculate that the modulation of the relative positions of S0 and S4 by β subunits could mechanistically explain their effects on voltage-dependent activation.